UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

Amended Date September 29, 2010
Original Date August 4, 2010

MEMORANDUM

Subject: Efficacy Review for 777-99, Brace
DP Barcode: 377492

From: Tajah Blackburn, PhD. Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

9/29/10

To: Jacqueline Campbell PM 34/ Killian Swift
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
Morris Corporate Center IV
399 Interspace Parkway
Parsippany, NJ 07054

Formulation from the Label:

Active Ingredient(s)	% by wt.
Alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) Dimethyl Benzyl Ammonium Saccharinate	0.10%
Ethanol	58.00%
Other Ingredients	41.90%
Total	100.00%

I BACKGROUND

The product, BRACE (EPA Reg. No. 777-99), is an EPA-approved disinfectant (bactericide, tuberculocide, fungicide, virucide), sanitizer, mildewcide, and deodorizer for use on hard, non-porous surfaces in household, institutional, commercial, food preparation, animal care, and hospital or medical environments. The applicant requested to amend the registration of this product to add new claims for effectiveness as a disinfectant against Avian influenza A (H5N1) virus, Swine influenza A (H1N1) virus, and Pandemic 2009 H1N1 Influenza A virus in addition to adding claims for effectiveness as against *Pseudomonas aeruginosa* on soft surfaces (cotton and synthetic fabrics). The current review addresses the registrant's rebuttal to the Efficacy Review, dated August 4, 2010. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated April 14, 2010), EPA Form 8570-35 (Data Matrix), five studies (MRID 480663-02 through 480663-06), Statements of No Data Confidentiality Claims for all five studies, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: appliances, bathtubs, bed frames, bed springs, bidets, blinds, cabinets, cages, chairs, changing tables, clean-up carts, counters, cribs, cuspidors, desks, diaper pails, dish pails, doorknobs, drains, dressing carts, drinking fountains, examination tables, faucets, fixtures, floors, furniture, garbage cans, garbage pails, highchairs, kennels, lamps, laundry hampers, light switches, linen carts, litter boxes, mattress covers, mirrors, outdoor patio furniture, pens, recycling bins, remote controls, salad bar sneeze guards, showers, sinks, sports equipment, stretchers, tables, telephones, toilets, tools, toys, urinal exteriors, walls, wheelchairs, whirlpool interiors, and windows. The proposed label indicates that the product may be used on hard, non-porous surfaces including: crystal, enamel, glass, glazed ceramic, glazed porcelain, glazed tile, laminated surfaces, linoleum, marble (synthetic), Marlite, metal (i.e., brass, chrome, copper, stainless steel, tin), Parquet, plastic, sealed granite, and vinyl. Directions on the proposed label provide the following information regarding use of the product as a disinfectant and spot soft surface sanitizer:

Disinfectant: Pre-clean surfaces prior to use. Hold container upright 6-8 inches from surface. Spray 2 to 3 seconds until covered with mist. Let stand for 30 seconds when treating against Avian influenza A (H5N1) virus, Swine influenza A (H1N1) virus, and Pandemic 2009 H1N1 Influenza A virus. Allow to air dry.

Spot Soft Surface Sanitizer: Spray until fabric is wet. Do not saturate. Fabric must remain wet for 30 seconds. Let air dry. For difficult odors or heavy fabrics, repeat application.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Spot Soft Surface Sanitization

This study is designed to evaluate the antimicrobial efficacy of spray application sanitizers on pre-cleaned or lightly soiled, non-food contact soft surfaces. For sanitizer products intended for use on soft, non-food contact surfaces, a fabric carrier method is used to generate efficacy data. The test system proposed is a modification of the ASTM approved method for the evaluation of the antimicrobial efficacy of sanitizers on non-food contact surfaces. The method is modified for spray product application.
A film of bacterial cells, dried on fabric carriers, is exposed to the test substance for a specified contact time. After exposure, the carriers are transferred to vessel containing neutralizing subculture media and assayed for survivors. Appropriate viability and sterility of organism population and neutralization controls are performed. Carrier type claimed on the label must be consistent with the test system. The test material meets effectiveness requirements if the product kills an average of at least 99.9% (3 log reduction) of the required organism on the 6 replicate carriers. Controls must always meet the stipulated criteria.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 480663-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Virus: Avian influenza A (H5N1) virus, for Formula No. 1338-027 and Formula No. 1178-172, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – March 10, 2009. Project Number A07417.

This study was conducted against Avian influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG, CDC #2006719965; obtained from the Centers for Disease

Control, Atlanta, GA), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Formula No. 1338-027, Lot No. 1325-045; and Formula No. 1178-172, Lot No. 1325-046) of the product were tested according to ATS Labs Protocol No. REK01021209.AFLU (copy provided). The product was received ready-to-use, as an aerosol spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 23.0°C at 18.9% relative humidity. For each lot of product, separate dried virus films were sprayed (3 seconds) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 21.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

2. MRID 480663-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Virus: Swine influenza A (H1N1) virus, for Formula Nos. 1178-172, 1338-027, and 1338-016, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 4, 2009. Project Number A07713.

This study was conducted against Swine influenza A (H1N1) virus (Strain A/Swine/Iowa/15/30; ATCC VR-333), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Three lots (Formula No. 1178-172, Lot No. 1325-069; Formula No. 1338-027, Lot No. 1325-047; and Formula No. 1338-016, Lot No. 1367-073) of the product were tested according to ATS Labs Protocol No. REK01042709.SFLU.1 (copy provided). The product was received ready-to-use, as an aerosol spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (3 seconds) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified

cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed.

3. MRID 480663-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Virus: 2009-H1N1 Influenza A virus, for Formula No. 1338-016 and Formula No. 1338-027, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – November 19, 2009. Project Number A08498.

This study was conducted against 2009-H1N1 Influenza A virus (Strain A/Mexico/4108/2009, CDC# 2009712192; obtained from the Centers for Disease Control, Atlanta, GA), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Formula No.1338-016, Lot No. 1367-073; and Formula No. 1338-027, Lot No. 1325-047) of the product were tested according to ATS Labs Protocol No. REK01100509.FLUA.1 (copy provided). The product was received ready-to-use, as an aerosol spray. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 22.0°C at 26.0% relative humidity. For each lot of product, separate dried virus films were sprayed (3 seconds) until thoroughly wet with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 22.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

4. MRID 480663-05, "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Surface Non-Food Contact Surfaces (Modification for Spray Product Application)" by Jill Ruhme. Study Completion Date—January 8, 2009. Study Identification Number A06890.

The study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Two lots (Lots Nos. 1325-046 and 1325-045) for Formula Numbers 1178-172 and 1338-027, respective to lot numbers, were tested using ATS Protocol Number REK0109080.NFS.2 (protocol attached). Both lots were ≥ 60 days old at the time of testing. Testing was conducted in the presence of 5% organic soil load. Each sterile synthetic carrier was inoculated with 30 µl of the 48±4 hour culture. The inoculated carriers were dried at 35-37°C at a humidity of 39% for 20 minutes. After the drying procedure, all the plates were removed to room temperature. The first inoculated carrier

was sprayed with the test substance for 2-3 seconds (sprayed for 3 seconds in test) at a distance of 6-8 inches above the carrier surface. All remaining carriers were sprayed using staggered intervals. All carriers were held at room temperature for a 30 second exposure period. Five carriers and 3 control carriers were treated for each batch tested. Following the 30 second exposure, the fabric carriers were placed into 20 ml of the appropriate neutralizer solution. Following the neutralization of the carriers, the excess liquid from the each plastic Petri dish was transferred to the appropriate neutralizer vessel containing the analogous carrier. The jars were rotated vigorously on an even plane approximately 50 rotations to suspend the surviving organisms in the neutralizer solution. Each carrier continued to be neutralized, using identical staggered intervals and agitating each in turn. Within 30 minutes after neutralizing the carriers, 1.00 ml of 10^0 and 10^{-1} dilutions of the neutralizer solution was plated from each of the jars in duplicate using standard spread plate techniques and TSA with 5% sheep blood. Plates were incubated at 35-37°C for 48±4 hours prior to observation for number of colonies. Controls included those for carrier quantitation, neutralization confirmation, purity, sterility, and inoculum count.

Note: Protocol deviations/amendments reported in the study were reviewed.

4. MRID 480663-06, "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Surface Non-Food Contact Surfaces (Modification for Spray Product Application)" by Jill Ruhme. Study Completion Date—January 8, 2009. Study Identification Number A06891.

The study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Two lots (Lots Nos. 1325-046 and 1325-045) for Formula Numbers 1178-172 and 1338-027, respective to lot numbers, were tested using ATS Protocol Number REK0109080.NFS.1 (protocol attached). Both lots were ≥ 60 days old at the time of testing. Testing was conducted in the presence of 5% organic soil load. Each sterile fabric (cotton) carrier was inoculated with 30 µl of the 48±4 hour culture. The inoculated carriers were dried at 35-37°C at a humidity of 41% for 20 minutes. After the drying procedure, all the plates were removed to room temperature. The first inoculated carrier was sprayed with the test substance for 2-3 seconds (sprayed for 3 seconds in test) at a distance of 6-8 inches above the carrier surface. All remaining carriers were sprayed using staggered intervals. All carriers were held at room temperature for a 30 second exposure period. Five carriers and 3 control carriers were treated for each batch tested. Following the 30 second exposure, the fabric carriers were placed into 20 ml of the appropriate neutralizer solution. Following the neutralization of the carriers, the excess liquid from the each plastic Petri dish was transferred to the appropriate neutralizer vessel containing the analogous carrier. The jars were rotated vigorously on an even plane approximately 50 rotations to suspend the surviving organisms in the neutralizer solution. Each carrier continued to be neutralized, using identical staggered intervals and agitating each in turn. Within 30 minutes after neutralizing the carriers, 1.00 ml of 10^0 and 10^{-1} dilutions of the neutralizer solution was plated from each of the jars in duplicate using standard spread plate techniques and TSA with 5% sheep blood. Plates were incubated at 35-37°C for 48±4 hours prior to observation for number of colonies. Controls included those for carrier quantitation, neutralization confirmation, purity, sterility, and inoculum count.

Note: Protocol deviations/amendments reported in the study were reviewed.

V RESULTS

Cotton Fabric--*Pseudomonas aeruginosa* 5.62×10^6

Batch 1325-045 (≥60 days old)					
Organism	Carrier	#Survivors/carrier	Average Log_{10}	Geometric Mean	Percent Reduction
Pseudomonas aeruginosa	1-5	$<2 \times 10^1$	1.80	6.31×10^1	>99.9%
Batch #1325-046 (≥60 days old)					
Pseudomonas aeruginosa	1-5	$<2 \times 10^1$	<1.78	$<6.03 \times 10^1$	>99.9%

Synthetic Fabric--*Pseudomonas aeruginosa* 1.23×10^7

Batch 1325-045 (≥60 days old)					
Organism	Carrier	#Survivors/carrier	Average Log_{10}	Geometric Mean	Percent Reduction
Pseudomonas aeruginosa	1-5	$<2 \times 10^1$	<1.30	$<2.00 \times 10^1$	>99.9%
Batch #1325-046 (≥60 days old)					
Pseudomonas aeruginosa	1-5	$<2 \times 10^1$	<1.30	$<2.00 \times 10^1$	>99.9%

MRID Number	**Organism**	**Results**			**Dried Virus Count**
			Lot No. 1325-045	**Lot No. 1325-046**	
480663-02	Avian influenza A (H5N1) virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	**Organism**	**Results**				**Dried Virus Count**
			Lot No. 1325-069	**Lot No. 1325-047**	**Lot No. 1367-073**	
480663-03	Swine influenza A (H1N1) virus	10^{-1} to 10^{-6} dilutions	Complete inactivation			$10^{5.7}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
480663-04	2009-H1N1 Influenza A virus	10^{1} to 10^{-7} dilutions	---	Complete inactivation		$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

VI CONCLUSIONS

1. The submitted efficacy data support the use of the product as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 30-second contact time:

Avian influenza A (H5N1) virus	MRID 480663-02
Swine influenza A (H1N1) virus	MRID 480663-03
2009-H1N1 Influenza A virus	MRID 480663-04

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

2. The submitted efficacy data support the use of the product as a spot soft surface sanitizer against *Pseudomonas aeruginosa* on cotton (MRID No. 480663-06) and synthetic (MRID No. 480663-05) soft surfaces in the presence of 5% organic soil load for a 30-second contact time. Dried carrier counts were 5.62×10^6 CFU/carrier and 1.23×10^7 CFU/carrier. A 3-log reduction was observed for *Pseudomonas aeruginosa* utilizing each carrier type.

VII RECOMMENDATIONS

1. The proposed label claims are acceptable regarding the use of the product, BRACE, as a disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 30-second contact time:

Avian influenza A (H5N1) virus
Swine influenza A (H1N1) virus
Pandemic 2009 H1N1 Influenza A virus

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claim is acceptable regarding the use of the product, BRACE, as a spot soft surface sanitizer against *Pseudomonas aeruginosa* on pre-cleaned, cotton surfaces for a contact time of 30 seconds. The allowable spray distance and spray time is provided in the Directions for Use.

3. The proposed label claim is unacceptable regarding the use of the product, BRACE, as a spot soft surface sanitizer against *Pseudomonas aeruginosa* on pre-cleaned, synthetic surfaces for a contact time of 30 seconds. The registrant needs to describe what includes synthetic surfaces consistent with the test system. On the proposed label, silk, rayon acetate, and satin are fabric types for which the product cannot be used. To this point, elucidation of the fabric type for which data was provided is necessary for clarification on the proposed label. In the September 2008 efficacy review, information was provided regarding the carrier types (polyester/acrylic fabrics). The Agency is requesting the same information on the proposed label.

4. The following revisions to the proposed label are required:

   - The claim "fast" has not been quantitated by the Agency. Until such time, these claims are unacceptable.
   - With some contact time in excess of 30 seconds, the claim "quick dry formula" conflicts with longer contact times.
   - Soft surface sanitization claims must be limited to "spot" soft surface sanitization claims. Consistent with the September 2008 efficacy review; briefly "claims throughout the proposed label imply use of the product on large soft surface areas, not "spot treatment" of surface areas suggested in the Agency's response to Item# 1 in the letter dated June 10, 2008. The proposed label must be revised, as appropriate".
   - Remove the claims, "Kills all major types of Flu Viruses", "Kills the Cold Virus", "Kills the #1 cause of the Cold and Flu Viruses" and "Kills the Rhinovirus, the leading cause of the common cold" as these claims are false and misleading. The information provided references the Agency's usage of the Emerging Pathogens and Disinfection Hierarchy for Antimicrobial Products. This guidance proposes to utilize an organism hierarchy to identify effective products for use with emerging pathogens and to permit registrants to make limited statements against such pathogens. Usage of this document is limited to incidences when novel emerging and re-emerging pathogens for which testing has not been conducted emerge.